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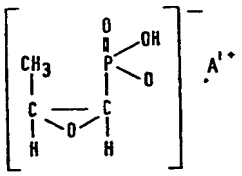
(54) Derivatives of (—)-*cis*-1,2-Epoxypropylphosphonic Acid, Their Preparation, and Pharmaceutical Compositions Containing Them

(57) Derivatives of (—)-*cis*-1,2-epoxypropylphosphonic acid ("phosphonomycin" or "Fosfomycin") having antibacterial chemotherapeutic properties and of use in human therapy, e.g. as pharmaceutical compositions, are of the general formula:



in which A'^+ is cation of an aminoacid, as for example lysine, arginine, ornithine, cystine, methionine, glycine, citrulline, etc., or of a naturally occurring trimethylammonium derivative, as for example choline, carnitine, betaine, etc., and can be prepared by reaction between substantially equimolecular quantities of (—)-*cis*-1,2-epoxypropylphosphonic acid and the aminoacid or of trimethylammonium derivative, preferably in an aqueous solution, or in a mixture of water and an alcohol such as methyl or ethyl alcohol.

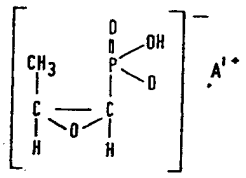
GB 2 013 682 A

SPECIFICATION

Derivatives of (—)-*Cis*-1,2-Epoxypropylphosphonic Acid, Their Preparation, and Pharmaceutical Compositions Containing Them

The present invention relates to derivatives of (—)-*cis*-1,2-epoxypropylphosphonic acid ("phosphonomycin" or "fosfomycin") having antibacterial chemotherapeutic properties and of use in human therapy.

The present invention is concerned with compounds of the general formula:



in which A'^{+} is cation of an aminoacid, as for example lysine, arginine, ornithine, cystine, methionine, glycine, citrulline, etc, or of a naturally occurring trimethylammonium derivative, as for example choline, carnitine, betaine, etc.. The compounds are therefore salts of (—)-*cis*-1,2-epoxypropylphosphonic acid with aminoacids or trimethylammonium derivatives.

According to the invention, the preparation of the compounds can take place by reaction between substantially equimolecular quantities of (—)-*cis*-1,2-epoxypropylphosphonic acid and the aminoacid or trimethylammonium derivative, preferably in an aqueous solution, or in a mixture of water and an alcohol such as methyl or ethyl alcohol.

Aminoacids of the L or D series or racemic compounds can, of course, be used.

The L-lysine and L-arginine salts of (—)-*cis*-1,2-epoxypropylphosphonic acid are already known but their superior and unforeseeable therapeutic properties have never hitherto been described. With these two specific exceptions the compounds are new and are claimed *per se*.

The invention also has for its subject a medicine i.e. pharmaceutical composition which contains as its active principle phosphonomycin salified with an aminoacid such as, for example lysine, arginine, ornithine, cystine, etc, or with a naturally occurring trimethylammonium derivative.

The active principle is generally associated with a therapeutically administrable medium.

Illustrative Examples are given below of the preparation of compounds of the invention.

Example 1.

Phosphonomycin salt with L-lysine

13.8 g of phosphonomycin in acid form was freshly prepared from a calcium or sodium salt thereof and dissolved in 50 cc of distilled water.

To this solution was added a solution containing 30 g of L-lysine dissolved in 30 g of water and 30 g of methanol. The solution was added with stirring and with control of the temperature of the mixture.

Agitation was continued for 30 minutes and then 50 ml of acetone followed by 50 ml of isopropyl alcohol and thereafter 50—100 ml of ethylether were added. In this manner an amorphous white precipitate was obtained which was pump-filtered and washed several times with ethyl ether.

Melting point: 165—189°C

Analysis for $C_9H_{21}N_2O_6P$ MW: 284

found:
C 38.20 H 7.42 N 9.85

theoretical:
C 38.03 H 7.39 N 9.86

Example 2

Phosphonomycin salt with L-arginine

A solution of 27.7 g of the (+) phenethylamine salt of phosphonomycin in 350 ml of distilled water was cooled to 0°C and percolated through an ion-exchange resin (Amberlite IR-120) which was in the H^+ form and previously cooled to 0—3°C.

The resin was washed with 350 ml of cold distilled water. The aqueous fractions eluted were pooled and treated with 17.4 g of L-arginine in 100 ml of water. Agitation for 15 minutes and then evaporation to dryness in a vacuum at low temperature gave a solid white residue which was pulped in methanol.

The desired product was thereby obtained, melting point 215—217°C with decomposition.

Analysis for $C_9H_{21}N_4O_6P$ MW: 312

found:
C 34.48 H 6.91 N 18.26

theoretical:
C 34.61 H 6.73 N 17.95

Example 3

Phosphonomycin salt with glycine

A solution of 27.7 g of the (+) phenethylamine salt of phosphonomycin in 350 ml of distilled water was cooled to 0°C and percolated through an ion-exchange resin (Amberlite IR-120) in the H^+ form and previously cooled to 0—3°C.

The resin was washed with 350 ml of cold distilled water and the eluted aqueous fractions pooled and treated with a cold solution of 7.5 g of glycine in 100 ml of water. Agitation for 15 minutes and evaporation to dryness in a vacuum at low temperature give a solid white residue which was dissolved in methanol, filtered through carbon, and reduced to a small volume in a vacuum at low temperature.

The desired product was obtained as a precipitate. It melted at 219—222°C with decomposition.

Analysis for $C_5H_{12}NO_6P$ MW: 213

found:
C 28.03 H 5.85 N 7.04

theoretical:
C 28.17 H 5.63 N 6.57

Example 4

Phosphonomycin salt with citrulline

A solution of 27.7 g of the (+) phenethylamine salt of phosphonomycin in 350 ml of distilled water was cooled to 0°C and percolated through an ion-exchange column (Amberlite IR-120) in the H^+ form, previously cooled to 0—3°C.

The resin was washed with 350 ml of cold distilled water. The eluted aqueous fractions were pooled and treated with 17.5 g of citrulline in 100 ml of water. The resultant mixture was agitated for 15 minutes, then dried in a vacuum at low temperature.

A solid white residue was obtained which was dissolved in methanol, filtered through carbon, and reduced to a small volume in a vacuum.

The desired product precipitated melting point 175—177°C.

Analysis for $C_9H_{20}N_3O_7P$ MW: 313

found:

C 34.80 H 6.21 N 13.22

theoretical:

C 34.51 H 6.39 N 13.42

Example 5

Phosphonomycin salt with L-carnitine

A solution of 27.7 g of the (+) phenethylamine salt of phosphonomycin in 350 ml of distilled water was cooled to 0—3°C and percolated through an ion-exchange column (Amberlite IR-120) in the H^+ form and previously cooled to 0—3°C.

The resin was washed with 350 ml of cold distilled water.

The eluted aqueous fractions were pooled and treated with 16.1 g of L-carnitine in 100 ml of water. The mixture was agitated for 15 minutes and then dried in a vacuum at low temperature.

A solid white residue was obtained which was dissolved in methanol, filtered through carbon, reduced to a small volume in a vacuum, and precipitated with isopropanol.

The desired product was thus obtained, it melted at 170—185°C with decomposition.

Analysis for $C_{10}H_{22}NO_7P$ MW: 299

found:

C 40.01 H 4.39 N 7.65

theoretical:

C 40.13 H 4.68 N 7.36

Example 6

Phosphonomycin salt with L-ornithine

A solution of 27.7 g of the (+) phenethylamine salt of phosphonomycin in 350 ml of distilled water was cooled to 0°C and percolated through an ion-exchange column (Amberlite IR-120) in the H^+ form and previously cooled to 0°C.

The resin was washed in 350 ml of cold distilled water. The eluted aqueous fractions were pooled and treated with 13.2 g of L-ornithine in 100 ml of water. After agitation for 15 minutes and then drying in a vacuum at low temperature, solid white residue was obtained which was dissolved in methanol, filtered through carbon, reduced to a small volume in a vacuum, and precipitated with isopropanol.

The desired product was thereby obtained, it melted at 190—192°C with decomposition.

Analysis for $C_8H_{19}N_2O_6P$ MW: 270

found:

C 35.27 H 7.16 N 10.53

theoretical:

C 35.56 H 7.04 N 10.37

Example 7

L-alanine salt of phosphonomycin

An aqueous solution of acid phosphonomycin prepared as explained in the above examples was mixed with a solution of 8.9 g of L-analine in 100 ml of water.

The solution was agitated for 15 minutes, dried by evaporation, and the residue recovered with ethanol. The residue obtained was the desired product and was collected by filtration.

Analysis for $C_6H_{14}NO_6P$ MW: 227

found:

C 31.58 H 6.32 N 6.25

theoretical:

C 31.72 H 6.17 N 6.17

Toxicology and Pharmacology

Toxicological and pharmacological results are discussed below for compounds of formula (I) and show an antibacterial action on germs sensitive to phosphonomycin which is equal to or superior to equimolecular doses of phosphonomycin with rapid absorption and higher concentration in the tissues.

Furthermore, when administered parenterally, the products of formula (I) appear free of any alteration of a cardiac-, circulatory-, or renal nature connected with the administration of large doses of other soluble phosphonomycin salts such as the sodium salt.

Toxicological Study

As examples, we find the products of Examples 1 (L-lysine salt) and 2 (L-arginine salt) have very low toxicity.

It was found that when administered intraperitoneally in the mouse the product of Example 1 shows an LD_{50} greater than 2 g/kg and the same is true for the product of Example 2.

When administered intravenously the product of Example 1 shows an LD_{50} in the mouse of 1.25 g while the product of Example 2 shows an LD_{50} of 1.40 g/kg.

In the dog, doses greater than 2.5 g/kg were administered intravenously without the appearance of toxic phenomena.

The LD_{50} for subcutaneous administration in the rabbit was demonstrated to be greater than 1 g/kg for the products of Examples 1 and 2.

In the rat it was possible to administer subcutaneously doses greater than 3 g/kg without the appearance of toxic phenomena.

Upon oral administration, whether to the rat or the mouse, the product of Example 1 or of Example 2 does not cause death or signs of toxicity even with doses of greater than 5 g/kg.

To evaluate chronic toxicity the test product was administered subcutaneously in the rat in the quantity of 150 mg/kg or 300 mg/kg daily for sixty-five consecutive days.

In these tests, measurement was made of the number of red corpuscles, the leukocytic formula, urine elimination, glycemia, azotemia, and transaminase before, during and after the treatment.

As compared with the control animals the treated animals showed no significant variation in the various parameters considered.

Intravenous administration in the dog of 1/kg of the product of Example 1 and of the product of Example 2 for twelve consecutive days also proved to be well tolerated having regard to the same biological parameters.

Tests performed on the guinea pig involving sensitizing the animal by the intradermal injection of 0.1 cc of a 10% solution of products of Examples 1 and 2 and then injecting intraveneously 4 cc/kg of the same solution twenty-one days after the intradermal injection did not bring on the appearance of sensitization phenomena, thus excluding allergic properties in the products of Examples 1 and 2.

The most favourable toxicity and tolerance aspects of the products of Examples 1 and 2 as compared with the disodium salt of phosphonomycin become evident through examination of the ECG, blood pressure, and renal elimination performed on animals treated acutely or chronically with the various phosphonomycin salts.

In the rat, slow intravenous perfusion with 100 mg/kg/min of the sodium salt of phosphonomycin leads after 15 minutes to the appearance in the ECG of abnormalities in the T wave and the S-T segment accompanied by extension of the QRS complex.

These alterations do not appear when administering the products of Examples 1 and 2 by the same method and containing doses of phosphonomycin itself equal to those present in the disodium salt.

Even more evident are the differences between the electrocardiographic alterations induced in the dog by slow perfusion of 200 mg/kg/min of disodium phosphonomycin or with equimolecular doses of the products of Examples 1 and 2. After 10 minutes, disturbances in rhythm and lengthening of the P-R section with irregularities in the T wave and the S-T section appear in animals treated with disodium phosphonomycin. In animals treated with the products of Examples 1 and 2 these irregularities, which are due to the large sodium load, do not appear.

Similar ECG irregularities are produced by sodium solutions containing the same quantity of sodium present in disodium phosphonomycin salt.

This finding confirms that it is not the phosphonomycin itself that is responsible for these toxic irregularities but its sodium salt.

Whereas disodium phosphonomycin solutions injected daily at 5 cc/kg as a 10% solution in to rats with one kidney removed cause the appearance after 20 days of hypertension in all animals, this does not happen with the injection of equimolecular quantities of the products of Examples 1 and 2 where after the same period of treatment no animal had hypertension and the first moderate hypertension signs appear only after 35 days of treatment and thereafter only in less than half of the animals.

Differences in the groups of animals can be ascertained also in urine evacuation which is not modified in animals treated with the products of Examples 1 and 2 in contrast with the result with prolonged administration of disodium phosphononmycin.

Pharmacological Trials

The antibacterial activity of the typical products of Examples 1 and 2, is similar to that of phosphonomycin if the concentrations are related to the quantity of active phosphonomycin.

The MIC and MPC calculated on the various bacterial strains sensitive to phosphonomycin appear to be analogous for the various phosphonomycin salts. The products of Examples 1 and 2 appear to be active against gram-positive and gram-negative bacterial strains. In particular, *Escherichia coli, Klebsiella pneumoniae, Salmonella. Staphilococcus aureus, Streptococcus pyogenes, Pseudomones aeruginosa, Serratia, Enterobacter,* and *Proteus* are sensitive to the action of the products of Examples 1 and 2.

To determine hematic levels, blood was drawn from the vena cava inferior of animals treated under light ethereal anesthesia and from the blood the serum was extracted.

To determine concentrations in tissues, organs (lungs, brain, kidneys, liver, heart) were removed in physiological solution, weighed and homogenized with a Turrax apparatus.

In accordance with conventional procedure, dilutions were made in buffered physiological solution of pH 7.4. Double dosage was made for each sample in Nutrient Agar Difco with the cup method using *Proteus vulgaris* 66/24 as the germ. Reading was made after 24 hours of incubation at 37°C.

In comparison with disodium phosphonomycin, both oral and intraperitoneal adminstration of the products of Examples 1 and 2 cause more rapid absorption and higher hematic concentrations.

Two hours after oral administration of 100 mg/kg of equimolecular doses of calcium phosphonomycin or of the products of Examples 1 and 2 the latter cause hematic concentrations 3—4 times higher. After intraperitoneal administration the concentrations of the products of Examples 1 and 2 are approximately double to that for the same period after administration of disodium phosphonomycin salt.

The difference diffusibility of the products of Examples 1 and 2 in the tissues is also quite apparent as compared with the other phosphonomycin salts such as disodium and calcium since after administration of an equal quantity of basic phosphonomycin (100 mg/kg) the products of Examples 1 and 2 administered orally give concentrations of more than double and for longer periods of time.

The concentrations of the products of Examples 1 and 2 appears to be particularly high in the liver, the kidneys, and the lungs.

The medicines of the invention are usable in human therapy for their antibiotic and antibacterial activity and can be formulated for administration either orally or parentally, due to their ease of solubilization. Formulation may be for their administration orally, intramuscularly, intraveneously, percutaneously e.g. topically or by aerosol, in the form for example of tablets, capsules, pills, phials, ointments, lotion, cream, or powders.

They can be associated with appropriate media or excipients.

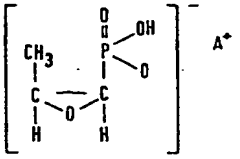
Indicatively but not restrictively some examples of pharmacological formulation of the products of Examples 1 and 2 are as follows:

Capsules, pills, or tablets containing 0.5 or 1g each of active product; a phial solution containing 1 g/cc of active product; a salve, cream or lotion containing 10—20% of active product in a pharmaceutically acceptable medium or excipient.

The doses of the product which can be administered daily vary according to therapeutic requirements e.g. from 1 or 2 capsules, pills or tablets 3 to 4 times a day to 1 or 2 phials containing 2 to 4 cc each intramuscularly, or 1 to 2 phials containing 5 to 10 cc intravenously or by slow perfusion 2 or more times a day, or topical application of ointment, cream or aerosolized solution 2 to 3 times a day or more often according to medical indications.

Claims

1. A salt of (—) *cis*-1,2-epoxypropylphosphonic acid of general formula (I):



(I)

in which A^+ represents the cation of an aminoacid (other than L-lysine or L-arginine) or a naturally occurring trimethylammonium compound.

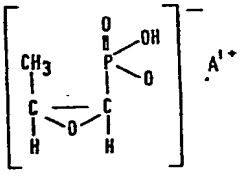
2. A salt according to claim 1 in which A^+ is the cation of ornithine, cystine, methionine, glycine, alanine, or of choline or betaine.

3. A salt according to claim 1 or 2 in which A^+ is the cation of an L-series or D series aminoacid.

4. A salt according to claim 1 or 2 in which A^+ is the cation of a racemic compound.

5. A process for the preparation of a salt as claimed in claim 1, wherein substantially equimolecular quantities or (—)-*cis*-1,2-epoxypropylphosphonic acid and a corresponding compound A are made to react in aqueous or aqueous/alcoholic solution.

6. A pharmaceutical composition with antibacterial chemotherapeutic activity which contains as its active principle one or more salts of (—)-*cis*-1,2-epoxypropylphosphonic acid of general formula (II)



(II)

in which A'^+ has the significance set forth above in claim 1 for A^+ or represents the cation of L-lysine or L-arginine.

7. A pharmaceutical composition according to claim 6 which contains as its active principle the (—)-*cis*-1,2-epoxypropylphosphonate of L-lysine.

8. A pharmaceutical composition according to claim 6 which contains as its active principle the (—)-*cis*-1,2-epoxypropylphosphonate of L-arginine.

9. The use in antibacterial chemotherapy of a salt of general formula (II) as defined in claim 6 or of a pharmaceutical composition as claimed in claim 6.

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